bio-protocol Preprint

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Protein expression and purification

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An abbreviated version of this protocol was published in eLIFE in Mar 2014

Structural basis of GSK-3 inhibition by N-terminal phosphorylation and by the Wnt receptor LRP6

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Detailed protocol

GSK3β 26-383/Axin 383-402

Wednesday, January 16, 2019

3:51 PM

GSK3β 26-383 in pET29b and Axin 383-402 in pGEX-TEV were cotransformed into BL21(DE3) RIL Codon-plus RIL cells (Stratagene). For growth, a single colony or a scraping from a glycerol stock was used to inoculate a 4 L culture in LB+ampicillin/kanamycin, which was grown overnight at 37°C with shaking. The culture was the induced with 0.1 mM IPTG at 16°C for 24 hours. The culture was then harvested by centrifugation at 6,774 x g for 15 minutes at 4°C, resuspended in 20 mL of 20 mM Tris pH 7.5, 300 mM NaCl, and 0.01% Triton X-100, and stored at -80°C for future use.

For purification, a 4 L pellet of GSK3β 26-383/GST-Axin 383-402 was defrosted, and protease inhibitors (200 µM benzamidine, 200 µM PMSF, 150 nM aprotinin, 1 µM E-64, and 1 µM leupeptin) and DNase (24 U; Sigma D4263) were added. The cells were then lysed using an Emulsiflex homogenizer (Avestin, Toronto, Canada) using two passes, with a target pressure of 15,000–20,000 psi. Protease inhibitors were added again as before, as well as EDTA to 1 mM, and the lysate was clarified by centrifugation at 38,000 x g for 30 minutes at 4°C. Protease inhibitors and EDTA were added as before, and the clarified lysate was bound to ~10 mL of glutathione-agarose () equilibrated in lysis buffer (20 mM Tris pH 7.5, 300 mM NaCl, 5% glycerol, 0.01% Triton X-100) by reduced-rate gravity flow. The flowthrough was bound again in the same manner, and the column was washed with 200 mL of wash buffer (20 mM Tris pH 7.5, 300 mM NaCl, 5% glycerol, 0.1% β-mercaptoethanol, 1 mM EDTA) at a reduced flowrate. The column was the eluted using 6x 5 mL fractions of elution buffer (20 mM Tris pH 7.5, 300 mM NaCl, 5% glycerol, 0.1% β-mercaptoethanol, 1 mM EDTA, 20 mM L-glutathione reduced). Peak fractions were identified using a BioRad 4-20% stain-free gel and detection with the BioRad gel imager. The two best fractions were pooled, and the GST tag was cleaved off by adding ~1 mg of TEV and allowing the reaction to proceed undisturbed at 4°C overnight. The cleaved material was then desalted into wash buffer using PD-10 desalting columns (GE Healthcare, Fairfield, CT) equilibrated in wash buffer according to the manufacturer's protocol. The desalted protein was then re-applied to ~10 mL of clean glutathione-agarose equilibrated in wash buffer in two roughly equal fractions, which were collected. The column was then eluted with 6x 5 mL fractions of wash buffer. Fractions corresponding to largely pure GSK3β 26-383/Axin 383-402 were identified using a BioRad 4-20% stain-free gel and detection with the BioRad gel imager. Depending on the application, they were either used fresh or concentrated by dialysis against outside buffer (20 mM Tris pH 7.5, 200 mM NaCl, 5% glycerol, 2 mM DTT, 1 mM EDTA, 10-40% PEG35,000) at 4°C over several days.

GSK3β 26-383

Wednesday, January 16, 2019

2:50 PM

GSK3β 26-383 in pET29b was transformed into BL21(DE3) RIL Codon-plus RIL cells (Stratagene). For growth, a single colony or a scraping from a glycerol stock was used to inoculate a 4 L culture in LB+kanamycin, which was grown overnight at 37°C with shaking. The culture was the induced with 0.1 mM IPTG at 16°C for 24 hours. The culture was then harvested by centrifugation at 6,774 x g for 15 minutes at 4°C, resuspended in 20 mL of 20 mM Tris pH 7.5, 300 mM NaCl, and 0.01% Triton X-100, and stored at -80°C for future use.

For purification, a 4 L pellet of GSK3β 26-383 was defrosted, and protease inhibitors (200 µM benzamidine, 200 µM PMSF, 150 nM aprotinin, 1 µM E-64, and 1 µM leupeptin) and DNase (16 units; Sigma D4263) were added. The cells were then lysed using an Emulsiflex homogenizer (Avestin, Toronto, Canada) using two passes, with a target pressure of 15,000–20,000 psi. Protease inhibitors were added as before, and the lysate was clarified by centrifugation at 38,000 x g for 30 minutes at 4°C. Protease inhibitors were added as before, and the clarified lysate was batch-bound to 10 mL of TALON resin equilibrated in lysis buffer (20 mM Tris pH 7.5, 300 mM NaCl, 0.01% Triton X-100, 5 mM imidazole) for 1 hour at 4°C with rotation. After the flowthrough was drained, the column was washed successively with 200 mL of wash buffer (20 mM Tris pH 7.5, 500 mM NaCl, 5 mM imidazole, 1.25 mM DTT) and 50 mL of lysis buffer. Next, the column was batch-eluted by adding 10 mL of elution buffer (20 mM Tris pH 7.5, 200 mM NaCl, 150 mM imidazole, 1 mM DTT) and rotating the the column for 1 hour at 4°C. The eluate was then drained, and the column was rinsed washed with 10 mL of elution buffer. The combined eluate was then diluted to 100 mL in MonoS buffer A (20 mM Tris pH 7.5, 2 mM DTT, 1 mM EDTA), filtered through a 0.2 µm PES filter, and run on a MonoS 10/300 GL. The gradient was 0-50% MonoS buffer B (0 mM Tris pH 7.5, 1 M NaCl, 2 mM DTT, 1 mM EDTA) over 20 CV, then 50-100% MonoS buffer B over 5 CV. The ten most concentrated 1.5 mL fractions corresponding largely pure GSK3 26-383 were identified using a BioRad 4-20% stain-free gel and detection with the BioRad gel imager and were immediately run on a preparative-grade Superdex 75 26/60 at 2.2 mL/min, with the running buffer being 20 mM Tris pH 7.5, 200 mM NaCl, 2 mM DTT, and 1 mM EDTA. Fractions corresponding to clean GSK3β 26-383 were identified using a BioRad 4-20% stain-free gel and detection with the BioRad gel imager, pooled, and concentrated by dialysis against outside buffer (20 mM Tris pH 7.5, 200 mM NaCl, 5% glycerol, 2 mM DTT, 1 mM EDTA, 10-40% PEG35,000) at 4°C for several days until the desired concentration of GSK3 was used. The GSK3β 26-383 was then used fresh, or 100% glycerol was added to a final concentration of 40%, and the protein was stored at -20°C for future use.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Weis, W. I.(2019). Protein expression and purification. Bio-protocol Preprint. bio-protocol.org/prep149.
2. Stamos, J. L., Chu, M. L., Enos, M. D., Shah, N. and Weis, W. I.(2014). Structural basis of GSK-3 inhibition by N-terminal phosphorylation and by the Wnt receptor LRP6. eLIFE. DOI: 10.7554/eLife.01998